Reclassification of *Roseivirga seohaensis* (Yoon *et al.* 2005) Lau *et al.* 2006 as a later synonym of *Roseivirga ehrenbergii* Nedashkovskaya *et al.* 2005 and emendation of the species description

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The precise taxonomic position of *Roseivirga seohaensis* strain SW-152^T, formerly [*Marinicola seohaensis*], was established. On the basis of data from DNA–DNA reassociation experiments and phenotypic analysis, it is proposed that this strain should be considered as a later heterotypic synonym of *Roseivirga ehrenbergii* KMM 6017^T Nedashkovskaya *et al.* 2005. Emended descriptions for *Roseivirga ehrenbergii* and for the genus *Roseivirga* are also given.

The genus *Roseivirga* was proposed to accommodate Gram-negative, strictly aerobic, heterotrophic, pink-pigmented, non-motile marine bacteria that belong to the phylum *Bacteroidetes* (Nedashkovskaya *et al.*, 2005a). The strains of the first two described species of the genus, *Roseivirga ehrenbergii* and *Roseivirga echinicomitans*, were isolated from the green alga *Ulva fenestrata* and from the sea urchin *Strongylocentrotus intermedius*, respectively (Nedashkovskaya *et al.*, 2005a, b). Soon after the description of the second species of the genus *Roseivirga*, *R. echinicomitans*, was completed, Yoon *et al.* (2005) proposed a novel genus with the type species *Marinicola seohaensis*. A single strain of this species was found in a seawater sample from the Yellow Sea. The type strain of *Marinicola seohaensis* had a very close phylogenetic relationship with the recognized species of the genus *Roseivirga*, demonstrating 99.1–99.9% 16S rRNA gene sequence similarity. The sequence data and phenotypic characteristics of [*Marinicola seohaensis*] served as evidence for the suggestion of the placement of the genus *Marinicola* and the species *Marinicola seohaensis* in the genus *Roseivirga* and for the emended description of the genus *Roseivirga* (Nedashkovskaya *et al.*, 2005b). However, a formal inclusion of [*Marinicola seohaensis*] in the genus *Roseivirga* as *Roseivirga seohaensis* was made by Lau *et al.* (2006), together with the description of an additional species of the genus, *Roseivirga spongicola*. Due to the very close phylogenetic distances and the absence of data from DNA–DNA reassociation experiments between *R. seohaensis*, *R. ehrenbergii* and *R. echinicomitans*, the true taxonomic status of *R. seohaensis* has not been fully established.

In this paper, we report the results of a study of the phylogenetic, genomic and phenotypic characteristics of species of the genus *Roseivirga* and suggest that *R. seohaensis* is a later synonym of *R. ehrenbergii*.

Genomic DNA extraction, PCR and sequencing of 16S rRNA gene were performed according to procedures described previously (Kim *et al.*, 1998). The sequence data obtained were aligned with those of representative members of selected genera that belong to the phylum *Bacteroidetes* using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the models of Jukes & Cantor (1969) and the trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) method. Phylogenetic trees were also constructed using the maximum-likelihood and maximum-parsimony methods (Felsenstein, 1993). Bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequences of *R. ehrenbergii* KMM 6017^T and *R. echinicomitans* KMM 6058^T revealed that they formed distinct lineages within the genus *Roseivirga* with a 16S rRNA gene sequence similarity of 99.1%. *R. seohaensis* SW-152^T clustered with these strains and displayed values of gene sequence similarity with its closest relatives, *R. ehrenbergii* KMM 6017^T and *R. echinicomitans* KMM

6058^T, of 99.9 and 99.1 %, respectively (Fig. 1). 16S rRNA gene sequence similarity between *R. seohaensis* SW-152^T and *R. spongicola* UST030701-084^T was 96 %.

DNA was isolated according to the method of Marmur (1961) and the DNA G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The values for the DNA G+C contents of strains KMM 6017^T, SW-152^T and KMM 6058^T obtained in this study were 41.3, 40.7 and 41.1 mol%, respectively. DNA–DNA hybridization experiments were performed using the method of De Ley *et al.* (1970). The DNA–DNA relatedness levels between *R. seohaensis* SW-152^T and *R. ehrenbergii* KMM 6017^T and *R. echinomitans* KMM 6058^T were 91 and 52 %, respectively. From these results, it is clear that strains SW-152^T and KMM 6017^T belong to the same species according to the proposal of Wayne *et al.* (1987). Therefore, *R. seohaensis* is a later heterotypic synonym of *R. ehrenbergii*.

Phenotypic analysis was performed using methods described previously (Nedashkovskaya *et al.*, 2003a, b). API 20E, API 20NE, API ZYM (bioMérieux) and Microlog GN2 plates (Biolog) were also used for physiological and biochemical analyses, with the tests performed according to the manufacturers' instructions, except that the inoculum was prepared by suspending cells in a 1.5 % NaCl solution.

Gliding motility was determined as described by Bowman (2000). For pigment characterization, strains KMM 6017^T, KMM 6058^T and SW-152^T were grown on marine agar for 48 h at 28 °C. The absorption spectrum of pigments extracted using 7:2 acetoin:methanol (v/v) was determined between 300 and 700 nm with a UV spectrophotometer (CE 7250/7000 series; CECIL).

The physiological, biochemical and morphological characteristics of the *Roseivirga* strains are given in the species description and in Table 1. The results of the phenotypic examination showed that there were many common traits between strain SW-152^T and its closest relative, *R. ehrenbergii* (Table 1). Several features such as hydrolysis of aesculin and Tweens 40 and 80 and resistance to ampicillin and tetracycline were strain-dependent. The presence of flexirubin-type pigments was not found using the Fautz–Reichenbach (Fautz & Reichenbach, 1980) method or by using spectrophotometry in this study. Strain SW-152^T was described as unable to hydrolyse gelatin, but in this study gelatinase production was shown to occur using the procedure described by Smibert & Krieg (1994).

Consequently, on the basis of significant molecular, genotypic and phenotypic similarities between strain SW-152^T and *R. ehrenbergii* presented in this study, we propose that *Roseivirga seohaensis* SW-152^T is a later heterotypic synonym of *Roseivirga ehrenbergii*.

Emended description of *Roseivirga ehrenbergii*

Roseivirga ehrenbergii [eh.ren.ber'gi.i. N.L. gen. n. *ehrenbergii* of Ehrenberg, after the German biologist Christian Gottfried Ehrenberg (1795–1876), for his contribution to the development of microbiology].

The description is as given by Nedashkovskaya *et al.* (2005a) with the following additions. Cells range from 0.2 to 0.5 µm in width and from 2.1 to 4.0 µm in length. On marine agar, colonies are 1–4 mm in diameter and pink–orange-pigmented. Growth occurs at 4–40 °C, at pH 5.5–9.5 and with 1–8 % NaCl. Optimal growth is observed at 23–30 °C, at pH 7.0–8.0 and with 2–3 % NaCl. Hydrolysis of aesculin and Tweens 40, 60 and 80 is strain-dependent. Does not form acid from D-fructose, D-mannose, *myo*-inositol or sorbitol. According to Microlog GN2 (Biolog), the type strain KMM 6017^T utilizes glycogen, L-arabinose, methyl pyruvate, α-ketoglutaric acid, glucuronamide, alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, L-histidine, L-ornithine, L-proline, L-serine, L-threonine and putrescine but does not utilize α-cyclodextrin, dextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabitol, cellobiose, *i*-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, α-lactose, α-D-lactose lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl α-D-glucoside, psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid, D-galacturonic acid,

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of the type strains of the genus *Roseivirga* and selected other representative taxa belonging to the phylum *Bacteroidetes*. Numbers at nodes indicate levels of bootstrap support (%) using 1000 resampled datasets. The same tree topology was also recovered from the maximum-likelihood and maximum-parsimony trees (not shown). Bar, 0.1 substitutions per nucleotide position.

Table 1. Phenotypic characteristics that differentiate species of the genus *Roseivirga*

Taxa: 1, *R. ehrenbergii* KMM 6017^T; 2, *R. ehrenbergii* SW-152 (= *R. seohaensis* SW-152^T); 3, *R. echinicomitans* KMM 6058^T; 4, *R. spongicola* UST030701-084^T. All taxa gave positive results for the following: respiratory metabolism; oxidase, catalase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, alkaline and acid phosphatase activities; growth at 1–8 % NaCl and at 31 °C; and hydrolysis of gelatin. All strains gave negative results for the following: flexirubin production; H₂S and indole production; hydrolysis of agar, casein, starch, cellulose (CM-cellulose and filter paper) and chitin; acid production from L-arabinose, L-fucose, D-galactose, D-glucose, D-lactose, maltose, melibiose, L-rhamnose, L-sorbose, sucrose, cellobiose, raffinose, DL-xylose, N-acetylglucosamine, glycerol, adonitol, inositol, mannitol, sorbitol, citrate; utilization of D-lactose, D-mannose, inositol, sorbitol and mannitol; α -glucuronidase and α -fucosidase activities; and susceptibility to kanamycin. +, Positive; –, negative.

Characteristic	1	2	3	4
Gliding motility	–	+	–	+
Nitrate reduction	–	–	+	–
Acetoin production	–	–	–	+
Growth at 37 °C	+	+	–	+
Hydrolysis of:				
DNA	+	+	–	+
Tween 20	+	+	–	+
Tween 80	–	+	–	+
Acid from amygdalin	–	–	+	–
Assimilation of:				
Glucose, N-acetylglucosamine, maltose, adipate	–	–	+	–
Enzyme activity:				
α - and β -Galactosidases	–	–	+	–
β -Glucosidase	–	–	+	+
Lipase (C14)	+	+	–	+
Susceptibility to:				
Ampicillin	+	–	+	–
Benzylpenicillin, streptomycin	–	–	+	–
Tetracycline	+	–	+	–
DNA G + C content (mol%)	41.3	41.1	40.7	43.7

D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, D-alanine, glycyl L-glutamic acid, hydroxy-L-proline, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, 2-aminoethanol, 2,3-butanediol, glycerol, α -DL-glycerol phosphate, glucose 1-phosphate or glucose 6-phosphate. Susceptible to carbenicillin, chloramphenicol, lincomycin and oleandomycin and resistant to benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. Resistance to ampicillin and tetracycline is strain-dependent. Fatty acids accounting for more than 1.0 % of the total are iso-C_{15:1}G (20.5–34.2 %), iso-C_{15:0} (24.0–33.5 %), iso-C_{17:0} 3-OH (7.7–11.2 %), iso-C_{16:0} 3-OH (4.1–7.6 %), iso-C_{15:0} 3-OH (3.0–5.6 %), iso-C_{13:0} (3.2–5.2 %), anteiso-C_{15:0} (2.4–4.5 %), C_{16:0} 3-OH (1.6–1.8 %), anteiso-C_{15:1} (0–1.8 %), iso-C_{16:0} (1.1–1.2 %), C_{15:0} (0.9–1.1 %) and

summed feature 3 (1.7–4.8 %), comprising C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH. Polar lipids of strain SW-152 are phosphatidylethanolamine, diphosphatidylglycerol, an unidentified phospholipid and a ninhydrin-positive lipid. The G + C content of the DNA is 40–41 mol%.

The type strain, KMM 6017^T (=KCTC 12282^T=LMG 22567^T), was isolated from the green alga *Ulva fenestrata* collected in Pallada Bay, Gulf of Peter the Great, East Sea.

A reference strain, SW-152 (=JCM 12600=KCTC 12312; the type strain of *Roseivirga seohaensis*), was isolated from a seawater sample in the Yellow Sea.

Emended description of the genus *Roseivirga* Nedashkovskaya *et al.* 2005

Roseivirga (Ro.se.i.vir'ga. L. adj. *roseus*, -a, -um pink-coloured; L. fem. n. *virga* rod; N.L. fem. n. *Roseivirga* a pink-coloured and rod-shaped marine bacterium).

The description is as given by Nedashkovskaya *et al.* (2005a), with the following additions. Rod-shaped cells

that can move by gliding. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase-negative. The main cellular fatty acids are straight-chain unsaturated and branched-chain unsaturated fatty acids iso-C_{15:1}, iso-C_{15:0}, iso-C_{15:0} 3-OH and iso-C_{17:0} 3-OH.

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